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Genital Mucosal Transmission of Simian Immunodeficiency Virus: Animal Model for Heterosexual Transmission of Human Immunodeficiency Virus

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An animal model for the heterosexual transmission of human immunodeficiency virus (HIV) was developed by the application of simian immunodeficiency virus (SIV) onto the genital mucosas of both mature and immature, male and female rhesus macaques. Virus preparations were infused into the vaginal vaults or the urethras (males) of the animals through a soft plastic pediatric nasogastric feeding tube. The macaques that were infected by this route (six males and nine females) developed SIV-specific antibodies, and SIV was isolated from peripheral mononuclear cells of all seropositive animals. One male and one female infected by this route developed severe acquired immunodeficiency syndrome-like disease with retroviral giant-cell pneumonia. As few as two inoculations of cell-free SIV containing 50 50% tissue culture infective doses induced persistent viremia. Cell-free virus preparations were capable of producing infection by the genital route. Much higher doses of virus were required to transmit SIV by this route than are required for transmission by intravenous inoculation. Thus, it appears that the mucous membranes of the genital tract act as a barrier to SIV infection. Spermatozoa and seminal plasma were not required for the genital transmission of SIV. Rarely, SIV was recovered from mononuclear cells in semen and vaginal secretions. The SIV-rhesus macaque model is suitable for assessing the role of cofactors in heterosexual transmission of HIV and will be useful for testing the effectiveness of spermicides, pharmacologic agents, and vaccines in preventing the heterosexual transmission of HIV.

Human immunodeficiency virus (HIV) is transmitted by both homosexual and heterosexual contact; however, the precise mechanism of sexual transmission is not clearly defined (35, 36). HIV must be transmitted via the mucosal surfaces of the genital tract, but it is not known whether spermatozoa, mononuclear cells, or seminal plasma enhances transmission or whether disruption of the genital epithelium is required for infection. In addition, some individuals remain uninfected despite multiple exposures (29), whereas others have been infected after one exposure to HIV-infected semen (42). An animal model would help elucidate the mechanisms and dose of virus required for transmission and provide a system for testing pharmacologic and biologic cofactors that may affect HIV transmission. For instance, do oral contraceptives enhance HIV sexual transmission, as was suggested by a recent study (37), and does the in vitro inactivation of HIV by spermicides (22) mean that spermicides are capable of preventing the sexual transmission of HIV? An animal model of the sexual transmission of HIV will also be important to determine whether vaccines can produce an immune response capable of preventing systemic infection after exposure of the genital mucosa to the virus.

HIV type 1 (HIV-1) has been inoculated into numerous animal species, including rhesus macaques (17, 18, 32). Chimpanzees become persistently viremic, without production of clinical disease (3). Chimpanzees have been used for limited studies on the sexual transmission of HIV. HIV-1 was inoculated onto the vaginal mucosa of one chimpanzee, producing viremia, but oral inoculation of HIV-1 failed to induce viremia in a second chimpanzee (15). However, an animal model for the sexual transmission of HIV would be more useful if disease symptoms were a feature of infection and if the model used a more available animal species.

Simian immunodeficiency viruses (SIV) are lentiviruses that are morphologically and genetically closely related to HIV. SIV has 75% genetic homology to HIV-2 (7, 13, 14). Like HIV, SIV exhibits T-cell tropism, using the CD4 molecule as a portion of its cellular receptor (28), and has an HIV-like genome organization (24). Intravenous inoculation of rhesus monkeys with SIV from rhesus macaques (SIV_{MAC}) or sooty mangabeys (SIV_{SM}) results in a uniformly fatal disease with the clinical signs and pathologic lesions of acquired immune deficiency syndrome (AIDS) (4–6, 8, 10, 11, 27, 30, 34). These isolates of SIV are closely related to one another genetically (23) and biologically (16, 34).

Approximately half of the animals intravenously inoculated with SIV die 3 to 12 months postinoculation (p.i.) with three or more of the following clinical signs and pathologic lesions: generalized lymphadenopathy, maculopapular rash, anemia, depressed helper/suppressor T-cell ratio, wasting, oral candidiasis, adenovirus-associated pancreatitis, adenovirus-associated colitis, retroviral pneumonia and/or encephalitis with giant cells, cryptosporidiosis, trichomoniasis, and disseminated cytomegalovirus infection (4–6, 8, 10, 11, 27, 30, 34). The other half of the intravenously inoculated animals survive 1 year or more but eventually develop a

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similar spectrum of disease (11). As with HIV infection in humans, there have been no reports of spontaneous recovery in SIV-infected macaques; all die eventually. Although no markers exist for predicting the development of specific opportunistic infections in SIV-infected macaques, the clinical course of the disease is very rapid in animals that fail to develop a strong antibody response 4 to 6 weeks after infection with SIV (11, 27, 49). The biologic and pathologic similarities between HIV infection in humans and SIV infection in macaques makes the SIV-rhesus macaque system an excellent model for studying the pathogenesis of AIDS in humans.

Although intravenous inoculation of SIV into macaques is an excellent model for studies of pathogenesis, this route of infection is not appropriate for study of the factors involved in the sexual transmission of HIV. To develop such a model, 6 male and 11 female macaques were inoculated with SIV via the genital mucosa. The results showed that simple application of SIV to the intact genital mucosa of mature and immature rhesus macaques is a useful model for the sexual transmission of HIV and that the disease induced by this route of inoculation was indistinguishable from that seen in intravenously inoculated animals. It was also shown that semen and seminal plasma were not essential cofactors for transmission of SIV to females.

MATERIALS AND METHODS

Animals. All animals used were colony-bred adult and juvenile rhesus macaques (*Macaca mulatta*) from the type D retrovirus-free and SIV-free colony at the California Primate Research Center. The animals were housed in accordance with American Association for Accreditation of Laboratory Animal Care standards. When necessary, the animals were immobilized with 1 mg of ketamine hydrochloride (Parke, Davis & Co., Morris Plains, N.J.) per kg injected intramuscularly. The investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Resource Council. Before use, the animals were negative for antibodies to HIV-2, SIV, type D retrovirus, and simian T-cell lymphotropic virus type 1.

SIV. All virus stocks were grown in human peripheral blood mononuclear cells (PBMC) because viral pathogenesis is readily preserved in these primary cell cultures. Extensive growth of SIV in T-cell lines results in greater virus yields but may attenuate the pathogenicity of SIV (5, 11). SIV_{MAC} grown in human PBMC was obtained from Ronald Desrosiers of the New England Primate Research Center. This stock was shown to cause persistent viremia and eventual disease in rhesus macaques at that facility (27). This SIV_{MAC} stock was expanded to approximately 100 ml (25 50% tissue culture infective doses [$TCID_{50}$]/ml) in human PBMC for the initial male transmission experiment and was shown to produce viremia and disease when inoculated intravenously into juvenile rhesus macaques at the California Primate Research Center. To further increase the supply of SIV_{MAC} for subsequent genital transmission studies, 300 ml of SIV_{MAC} was produced in human PBMC (50 $TCID_{50}$/ml). Cells and cell debris were removed by centrifugation at 650 × *g* for 10 min. All cell-free stocks was stored at −70°C without cryopreservatives. To ensure that the cell-free stocks did not contain residual cells, 2 ml of each stock was thawed and centrifuged at 1,500 × *g* for 10 min. No pellet was detected, but the fluid at the bottom of the tube was aspirated, placed on a slide, and stained with Wright Giemsa stain. No cells or cell debris was recognized when the slides were examined by light microscopy.

A cell-free SIV_{SM} stock (15 $TCID_{50}$/ml) was produced in human PBMC. The source of the initial isolate was a captive sooty mangabey in the United States (31). The cell-associated stock of SIV_{SM} was prepared from splenocytes of a rhesus macaque moribund with SIV_{SM}-induced simian AIDS. The cell-associated SIV_{SM} preparation was cryopreserved in 10% dimethyl sulfoxide and fetal calf serum and stored in liquid nitrogen. The stock contained 5.25×10^2 SIV-infected cells per ml.

The $TCID_{50}$ of the virus stocks were determined by using the modified MTT assay described previously (33). Briefly, serial twofold dilutions of the virus stocks were inoculated into replicates of six wells in a 96-well plate containing 10^4 CEMx174 cells (40) per well. The titers of the stocks were determined by the method of Reed and Muench (39), which uses 50% endpoints and involved scoring deaths (infected cells) and survivals (noninfected cells) over the entire dilution range. The absorbance values from each serial dilution were scored as infected or noninfected on the basis of the mean value of uninoculated cultures. Virus titers were confirmed by endpoint dilution in human PBMC, using a reverse transcriptase assay (31) to detect SIV infection in the cell cultures. There was no significant difference between the $TCID_{50}$ value of a stock when the assay was performed with CEMx174 cells or human PBMC. To determine the number of SIV_{SM}-infected cells, the proportion of infected cells in total viable cells was determined by endpoint dilution of infected cells. The infected cells were serially diluted 10-fold, and each dilution was cocultivated with human PBMC. A reverse transcriptase assay was used to detect SIV infection in the cocultivations (31).

Genital mucosal inoculation of females. The inoculum was infused through a 2.5-mm-outer-diameter (8 French) soft plastic pediatric nasogastric feeding tube (American Pharmaseal, Valencia, Calif.) into the vaginal vault of each immobilized female rhesus macaque. After each inoculation, the animals remained immobile for 15 min.

Genital mucosal inoculation of males. The inoculum was gently infused onto the urethral mucosa of the immobilized animals by inserting a 2.5-mm-outer-diameter (8 French) soft plastic pediatric nasogastric feeding tube (American Pharmaseal) approximately 1 cm into the urethral orifice. Although the animals remained immobile for 15 min, some of the inoculum drained from the urethra and came into contact with the skin surrounding the urethral orifice.

Intravenous inoculation of animals. To ensure that the cell-free SIV_{SM} and SIV_{MAC} stocks were pathogenic, they were inoculated into rhesus macaques. Two juvenile females (22657 and 22659) received cell-free SIV_{SM}, and two juvenile females (23217 and 23393) were inoculated with cell-free SIV_{MAC} (Table 1). Four juvenile males (22852, 23219, 22795, and 22816) received cell-free SIV_{MAC} (Table 1).

Virus isolation from peripheral blood, semen, and vaginal secretions. Virus was isolated from heparinized whole blood obtained from intravenously or genitally inoculated macaques. PBMC were separated by gradient centrifugation on Ficoll-Hypaque and washed in phosphate-buffered saline (PBS). The PBMC were stimulated for 72 h in RPMI 1640 containing 0.5 μg of staphylococcal enterotoxin A per ml and 10% fetal calf serum. PBMC (2×10^6 to 3×10^6) were cocultivated with 2×10^6 to 3×10^6 CEMx174 cells. The cultures were periodically examined for syncytium formation and supplied with fresh medium every 3 to 4 days. The

TABLE 1. Transmission of SIV to rhesus macaques by the genital and intravenous routes

Inoculation route and animal no.	Total no. of inoculations (dose)	Days p.i. for initial SIV isolation[a]
Females		
Vaginal mucosa		
21311	10[b]	14
21293	10[b]	37
8063	2 (15 $TCID_{50}$)[c]	Negative
17749	8 (50 $TCID_{50}$)[d]	7
16260	8 (50 $TCID_{50}$)[d]	14
17484	8 (5 $TCID_{50}$)[d]	14
18012	8 (5 $TCID_{50}$)[d]	Negative
23382, 23383, 22874, 23092	4 (50 $TCID_{50}$)[d]	14
Intravenous		
22657	1 (15 $TCID_{50}$)[c]	7
22659	1 (5 $TCID_{50}$)[c]	14
23217, 23393	1 (2.5×10^{-1} $TCID_{50}$)[d]	14
Males		
Urethral mucosa		
21875	3 (25 $TCID_{50}$)[d]	16
21943	3 (25 $TCID_{50}$)[d]	31
23221, 23227, 22950, 22953	4 (50 $TCID_{50}$)[d]	14
Intravenous		
22795, 22816	1 (2.5×10^{-3} $TCID_{50}$)[d]	14
22852, 23219	1 (2.5×10^{-1} $TCID_{50}$)[d]	14

[a] Each virus-positive animal was persistently infected as determined by multiple SIV isolations.
[b] Each inoculum was a 1-ml suspension of cell-free SIV_{SM} (15 $TCID_{50}$) mixed with 5.25×10^2 SIV_{SM}-infected PBMC.
[c] Each inoculum was a 1-ml suspension of cell-free SIV_{SM}.
[d] Each inoculum was a 1-ml suspension of cell-free SIV_{MAC}.

spent supernatant fluid was assayed for reverse transcriptase. In our laboratory, fresh CEMx174 cells and human PBMC have proven to be equally sensitive for the primary isolation of SIV from infected monkeys. PBMC collected from SIV_{SM}- and SIV_{MAC}-infected monkeys (intravenous inoculations) were divided and cocultivated with CEMx174 cells and human PBMC. Of the two cell types, the CEMx174 cell cultures showed changes consistent with SIV growth (cytopathic effects and Mg^{2+}-dependent reverse transcriptase activity) earlier and at a higher level than did the huPBMC. The CEMx174 cells are discarded after 3 to 4 months of culture, and cells preserved in liquid nitrogen are thawed and subsequently used.

A modification of the procedure described above was used to isolate SIV from the semen of SIV-infected male rhesus macaques. The ejaculate was obtained by electrostimulation (20) and allowed to liquefy by incubation at room temperature for 45 min. Seminal mononuclear cells were separated from the spermatozoa by dilution of the liquefied ejaculate in 20 ml of PBS and centrifugation at 72 × *g* for 10 min. The mononuclear cell pellet was resuspended in PBS, and the procedure was repeated four times or until about 95% of the spermatozoa were removed. The mononuclear cells were then cocultured with CEMx174 cells as described above. To culture virus from vaginal secretions of SIV-infected rhesus macaques, the vaginal vault was lavaged with PBS and the wash was collected. The mononuclear cells in the lavage were separated by gradient centrifugation on Ficoll-Hypaque, washed, and cultured for SIV as described above. To isolate SIV from the epididymis of 21893, the epididymis was collected at necropsy and tissue homogenates were cocultivated with CEMx174 cells as described above.

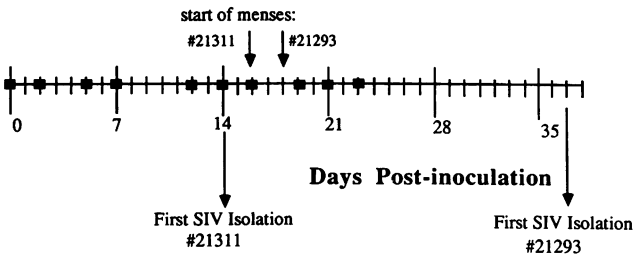

FIG. 1. Inoculation of females. Two mature female rhesus macaques were inoculated intravaginally with a mixture of cell-free SIV_{SM} (15 $TCID_{50}$) and cell-associated SIV_{SM} (5.25×10^2 SIV_{SM}-infected cells per ml). Each square represents one inoculation. Short arrows show when menses began, and long arrows indicate the first day that SIV was recovered from the PBMC of the inoculated animals.

Immunoblot analysis of serum. Serum from inoculated animals was analyzed for the presence of SIV-specific antibodies by Western immunoblot assay as previously described (31).

Collection and preparation of tissue samples. Tissues collected at necropsy were fixed in 10% buffered Formalin, embedded in paraffin, sectioned at 6 μm, stained with hematoxylin and eosin, and examined by light microscopy.

RESULTS

Transmission of cell-associated and cell-free SIV_{SM} to female rhesus macaques. To transmit SIV across the intact vaginal epithelium of female rhesus macaques, a mixture of cell-free and cell-associated SIV_{SM} was inoculated into the vaginas of two mature females (Fig. 1). A mixture of cell-free and cell-associated virus was used to maximize the possibility of achieving infection by the genital route. The animals were inoculated 10 times over a 4-week period (pre- and postmenses exposure for each female; Fig. 1). At 2-week intervals, samples of heparinized whole blood and serum were collected for virus isolation and immunoblot analysis. At 14 days p.i., the PBMC of female 21311 were virus positive and those of 21293 were negative (after five SIV inoculations) (Table 1 and Fig. 1). The PBMC of female 21293 did not contain detectable virus on day 29 after the complete series of 10 inoculations, but virus was recovered from the blood sample taken 8 days later on day 37 p.i. (Table 1 and Fig. 1). PBMC from both of the females were repeatedly cultured and remained SIV positive until euthanasia. Western blot analysis showed that both animals had detectable SIV-specific antibodies by 6 weeks p.i. (Fig. 2). The females were euthanized at 3 months p.i., at which time they had early lesions of simian AIDS, including generalized lymphadenopathy and splenomegaly. Histopathologic findings included mild multifocal lymphocytic interstitial pneumonia in both animals.

Transmission of cell-free SIV_{SM} to female rhesus macaques. In an attempt to produce infection with a small amount of cell-free virus, a third female (8063) was inoculated vaginally twice during the late follicular phase of the menstrual cycle (days 13 and 15; the first day of menstruation is day 1). A 1-ml inoculum containing cell-free SIV_{SM} (15 $TCID_{50}$) was used. Female 8063 did not become virus (Table 1) or antibody positive during a 10-month observation period. In contrast, a single intravenous inoculation of two females

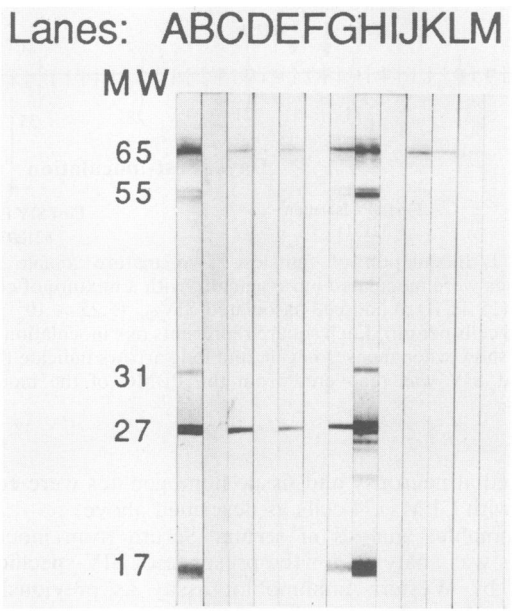

FIG. 2. Immunoblot analysis of serum from rhesus macaques infected with SIV via the intravenous (lane A) or genital mucous membrane (lanes B through M) route. Lanes: B and C, female 21311 at preinoculation and 6 weeks p.i., respectively; D and E, female 21293 at preinoculation and 6 weeks p.i., respectively; F through H, male 21875 at preinoculation and at 6 and 20 weeks p.i., respectively; I through M, male 21943 at preinoculation and at 6, 12, 20, and 25 weeks p.i., respectively. Animal 21943 mounted a weak response, mainly against the reverse transcriptase (p66), which steadily declined until death (lane M). The sera from the two males were tested as a single group so that relative strength of the antibody response could be estimated by comparing the intensities of the bands. Molecular size markers (in kilodaltons) are shown on the left. The virus antigen preparation used for this immunoblot contained an unusually small quantity of the transmembrane protein gp 32. The blots in this figure and those on Fig. 3 were done separately, and the virus antigen preparations were made at different times.

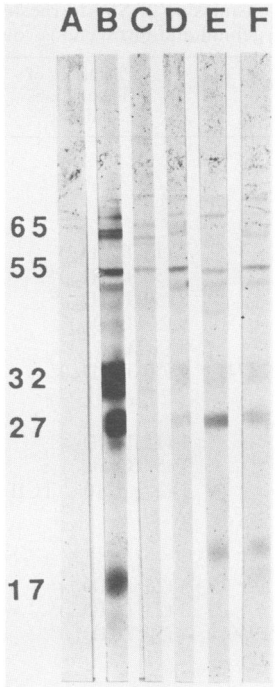

FIG. 3. Immunoblot analysis of serum from female rhesus macaques inoculated with cell-free SIV_{MAC} via the genital mucosa twice weekly for 4 weeks. Lanes: A and B, negative and positive control sera, respectively; C through F, 6-week p.i. sera from animals 18012, 17484, 16260, and 17749, respectively. Note that the animal that resisted mucosal challenge (18012; lane C) did not produce antibody to SIV and that the infected animals (17749, 16260, and 17484) were seropositive.

with 1 ml of the same stock of SIV_{SM} at different concentrations (15 and 5 $TCID_{50}$) induced persistent viremia in both animals (22657 and 22659) (Table 1). These data suggest that the dose of SIV required for mucosal transmission is considerably higher than the dose required for intravenous transmission.

Transmission of cell-free SIV_{MAC} to female rhesus macaques. To further determine the infectious dose of cell-free virus required for the genital transmission of SIV and to determine whether cell-free virus alone was capable of producing infection, four mature female rhesus macaques were inoculated with a cell-free stock of SIV_{MAC} that had a higher titer (50 $TCID_{50}$) than did the SIV_{SM} stock used previously. Two animals (17749 and 16260) received 1 ml of undiluted SIV_{MAC} virus stock, and two (17484 and 18012) received 1 ml of a 10-fold dilution (Table 1). The inoculum was infused into the vaginal vault of each animal eight times (twice a week for 4 weeks). PBMCs from the animals were cultured on days 2 (after one inoculation), 7 (after two inoculations), 14 (after four inoculations), 28 (after eight inoculations), and 60. SIV was isolated from the PBMC of the two females inoculated with the undiluted cell-free SIV_{MAC} on day 7 (17749) and day 14 (16260) (Table 1). Of the two females that received the 10-fold dilution of virus, one (17484) was positive on day 14 and the other (18012) had remained virus negative at 5 months p.i. (Table 1). Western blot analysis of serum from these four animals revealed that the three females that became viremic were seropositive for SIV-specific antibodies by 6 weeks p.i., whereas the one uninfected female (18012) had not seroconverted by 5 months p.i. (Fig. 3). Of the three infected females, all were viremic at 8 months p.i.; two are clinically normal, and the third (16260) has been euthanatized because of severe anemia and thrombocytopenia.

In addition to this group of adults, four juvenile female rhesus macaques (23382, 23383, 22874, and 23092) were vaginally inoculated with undiluted SIV_{MAC} (50 $TCID_{50}$) twice a week for 2 weeks (Table 1). All four of the animals were virus positive by 14 days p.i., and all were seropositive by 4 weeks p.i. At 6 months p.i., three of these animals were healthy. One (23383) was euthanatized because of hypoproteinemia, intermittent diarrhea, and weight loss. At necropsy, this animal had interstitial pneumonia and enterocolitis.

These data show that SIV can be readily transmitted across the intact vaginal epithelium of mature and immature rhesus macaques.

Transmission of cell-free SIV_{MAC} to male rhesus macaques. Two mature male rhesus macaques were exposed to 1 ml of cell-free SIV_{MAC} (25 $TCID_{50}$) once a week for 3 weeks (Fig. 4). After two inoculations (16 days p.i.), SIV was cultured from the PBMC of one male (21875), whereas the other (21943) did not have SIV-positive PBMC cultures until 31 days p.i., 2 weeks after completion of the series of three inoculations (Table 1). Each male has remained persistently viremic. Male 21875 had a strong antibody response to the

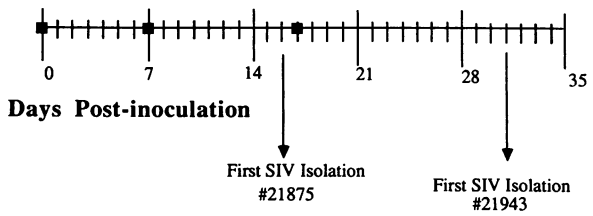

FIG. 4. Inoculation of males. The urethras of two mature male rhesus macaques were inoculated with 1 ml of human PBMC culture medium containing 25 $TCID_{50}$ of cell-free SIV_{MAC}. Each square represents one inoculation. Black arrows show the first day that SIV_{MAC} was isolated from PBMC.

p66, p55, p31, and p27 SIV antigens by 6 weeks p.i. (Fig. 2). This animal developed lymphadenopathy and splenomegaly but was alive and viremic at 10 months p.i. In contrast, male 21943 developed antibody only to the highly immunogenic p66 (12, 21) and a weaker response to the *gag* precursor p55 (12, 21). The antibody response to both of these antigens declined to a very low level by 20 weeks p.i. (Fig. 2). At 6 months p.i., 21943 became moribund with persistent diarrhea and severe weight loss. At necropsy, this animal had disseminated cytomegalovirus infection, ulcerative gastritis, and severe interstitial pneumonia with multinucleate giant cells (Fig. 5).

In addition to this group of adult males, four juvenile male rhesus macaques (23221, 23227, 22950, and 22953) were inoculated intraurethrally with cell-free SIV_{MAC} (50 $TCID_{50}$) twice a week for 2 weeks (Table 1). All were viremic by 14 days p.i. and seropositive by 4 weeks p.i. All four animals were healthy at 6 months p.i.

These experiments show that male and female rhesus macaques can be infected via the genital mucosa. Furthermore, the disease that eventually developed was indistinguishable from that seen in intravenously inoculated animals.

Isolation of SIV_{MAC} from semen and vaginal secretions. Paired semen and blood samples were obtained from each of two SIV-infected male rhesus. The blood samples served as positive controls for SIV infection. One animal had been infected via the genital mucosal route (21875), and the other (21893) had received an intravenous inoculation. Four semen and four blood specimens were collected over 4 months from 21875 and cultured as described. None of the semen specimens were SIV positive, but all four blood specimens were positive. This male was clinically normal at the time the samples were collected. Ten paired semen and blood specimens were taken over 8 months from the intravenously inoculated animal (21893). Two of ten semen specimens were SIV positive, whereas all ten blood specimens were positive. One of the virus-positive semen samples was collected 1 week before the animal was euthanatized because of its deteriorating clinical condition. SIV was also isolated from tissue homogenates made from the epididymis of this animal. The total number of mononuclear cells recovered from the ejaculates of these males ranged from 0 to 2×10^6 cells per ml. The samples from which virus was cultured contained approximately 10^6 cells per ml. Attempts to obtain a semen sample from the intraurethrally inoculated animal (21943) that developed severe simian AIDS were unsuccessful. The results from the two males studied (21875 and 21893) suggest that SIV is sporadically present in the mononuclear cells of semen. Twenty-one samples of vaginal secretions were taken from one intravenously inoculated female (7530) and cultured for SIV. Virus was isolated once from a sample collected at midcycle and once from a sample collected during menses. This animal was clinically normal at the time the samples were collected. These data suggest that SIV may be shed intermittently at any stage of the menstrual cycle.

Transmission of SIV after intravenous inoculation. All animals inoculated intravenously, whether with SIV_{MAC} or SIV_{SM}, became viremic by 14 days p.i. Of the six animals

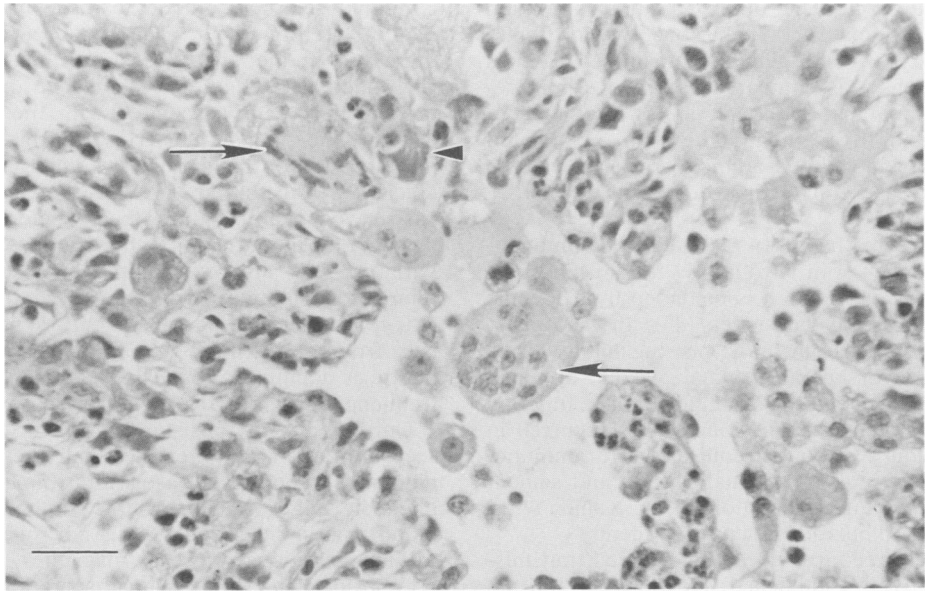

FIG. 5. Photomicrograph of the lung from male 21943 showing interstitial pneumonia. Note the presence of multinucleate giant cells (arrows) associated with SIV infection and a large cell with characteristic intracytoplasmic cytomegalovirus inclusions (arrowhead). Bar, 20 μm.

inoculated intravenously with cell-free SIV_{MAC}, one (22852) died with interstitial pneumonia with mutinucleate giant cells at 4 months p.i. Another (23393) died at 3 months p.i. with SIV encephalitis with giant cells, cryptosporidiosis of the gallbladder, jejunum, and pancreatic ducts and chronic typhlocolitis. And a third (23219) died at 8 months p.i., with interstitial pneumonia and gastroenteritis. The other three animals remain clinically normal; one (23217) has been infected for 6 months, and two (22795 and 22816) have been infected for 4 months.

DISCUSSION

In this study, multiple exposures of the genital tract of mature and juvenile, male and female rhesus macaques to SIV resulted in transmission across the genital mucosa. The disease produced in one of the genitally infected males (21943) was indistinguishable from the syndrome seen in rhesus macaques infected with SIV by the intravenous route. This animal developed only a weak antibody response that diminished as the infection progressed. This animal eventually was euthanatized because its poor condition and at necropsy had pathologic changes typical of severe AIDS-like disease. The other genitally infected animals have developed a strong antibody response, have been slow to develop clinical disease, and have experienced low mortality thus far. This correlation between a weak antibody response and a rapid clinical course has been previously reported for intravenously inoculated macaques (11, 26, 49).

In the first 6 months p.i., the six animals inoculated intravenously with cell-free SIV_{MAC} experienced relatively high morbidity and mortality. Three of the animals have been euthanatized with AIDS-like disease, and a third has severe clinical disease. In contrast, only 3 of the 13 male and female animals infected by genital inoculation with cell-free SIV_{MAC} have developed AIDS-like disease. One male (21943) with the lesions described above was euthanatized at approximately 5 months p.i. At 6 months p.i., one female (23383) with the lesions described above was euthanatized. One female (16260) was euthanatized because of severe anemia at 8 months p.i. The other 10 animals remain clinically normal, and 1 male (21875) has been viremic for 17 months. In summary, 3 of 13 genitally inoculated animals have developed disease, as compared with 3 of 6 intravenously inoculated animals. Although all of the infected animals are expected to eventually die with SIV-related disease, the genitally inoculated animals appear to have a more protracted disease course than do the intravenously infected macaques.

Human seminal plasma has been reported to be immunosuppressive in a number of in vitro assays (1, 26). Suppression of natural killer cell function is mediated by a specific prostaglandin (19-OH PGE) in semen (43). In macaques, there is a transient increase in blood prostaglandins levels after vaginal infusion of human seminal plasma, although there are no detectable changes in systemic immune function (2). In our study, genital transmission of SIV occurred in female rhesus macaques without a requirement for immunosuppressive cofactors such as seminal plasma and semen. Cell-free virus readily caused infection via the vagina without any additional manipulations or pretreatments.

The amount of cell-free virus needed for genital transmission was not definitively established. However, it appears that much more virus is needed to transmit SIV by the genital route than is required for transmission by the intravenous route. Two females received eight doses of cell-free SIV_{MAC}, each containing 50 $TCID_{50}$, four more females received four doses of cell-free SIV_{MAC}, each containing 50 $TCID_{50}$, and another two received eight doses of cell-free SIV_{MAC}, each containing 5 $TCID_{50}$. Of these eight animals, one resisted eight inoculations of 5 $TCID_{50}$ each (Table 1). The minimal animal infectious dose of intravenously inoculated cell-free SIV_{MAC} is about 2.5×10^{-3} $TCID_{50}$ (a single intravenous injection of two macaques with 1 ml of cell-free SIV_{MAC} containing 2.5×10^{-3} $TCID_{50}$ produced infection in both animals; Table 1). These data suggest that the minimal animal infectious dose by the mucosal route is much higher than the dose required for infection by intravenous inoculation. Further studies are in progress to determine whether a single genital inoculation of cell-free SIV_{MAC} can produce infection and the approximate dose required for transmission.

Although some degree of trauma undoubtedly occurs during normal sexual contact, for this animal model to be useful for understanding the sexual transmission of HIV, the inoculation procedure should not be overly traumatic to the genital mucosa. In women, HIV can be transmitted by infected semen infused into the vagina without trauma during artificial insemination (42). It is very unlikely that a small (2.5-mm-outer diameter), smooth plastic tube could cause significant trauma to the epithelium of the vagina during the inoculation procedure used in our study, and any damage produced was far less than occurs during normal mating. In the males, every attempt was made to avoid trauma during the inoculation procedure by gently infusing the inoculum onto the urethral mucosa, and there was no visible trauma to the urethra.

The finding that SIV is not consistently present in the seminal mononuclear cells of infected rhesus macaques may reflect individual variation in the number of mononuclear cells present in an ejaculate. There is wide variation among macaques in the number of leukocytes present in the semen, and an individual animal may have large fluctuations in the number of leukocytes present in semen samples collected at different times (C. Vandevoort, personal communication). There are large individual variations in the number of $CD4^+$ lymphoid cells and monocytes-macrophages in human semen (47). The number of mononuclear cells can be greatly increased in men with prostatitis (41), infertility (1), or reproductive tract infection (9). The increased number of mononuclear cells associated with these conditions could enhance the efficiency of HIV transmission. This variation may account for the fact that in some cases HIV seems to be efficiently transmitted through semen, whereas in other cases even years of unprotected heterosexual intercourse does not lead to infection of the female partner (29, 36). This variation may also explain the inconsistent results of attempts to isolate virus from the semen of HIV-infected men (25, 48). It will be necessary to culture seminal mononuclear cells from a large number of SIV-infected rhesus macaques before a definitive conclusion can be made regarding the frequency at which SIV is present in semen.

Our inability to consistently isolate SIV from the vaginal secretions of an SIV-infected female rhesus macaque despite numerous attempts (one isolation from a sample obtained at midcycle and one from a sample contaminated by menstrual blood) may indicate that SIV is not routinely present in the vaginal secretions of rhesus macaques. Attempts to isolate HIV from the cervicovaginal secretions of HIV-infected women have had variable results. In one study, it was possible to culture HIV from the cervical secretions of 4 of 14 infected women (44), and in another study HIV was

isolated from 4 of 8 infected women (46). In both of these studies, samples contaminated with menstrual blood were not cultured. In another study (45), virus was isolated from the cervical secretions of four of seven HIV-infected women. Blood and cervical secretions from these women were cultured at weekly intervals during a single menstrual cycle. No relationship between virus isolation and stage of menstrual cycle could be detected, and virus isolation from one sample (blood or cervical secretions) did not correlate with isolation from the other source. Thus, we are continuing to culture vaginal secretions, and we have begun to assay for SIV antigens in an attempt to define the frequency at which SIV is present in vaginal secretions of a large number of infected female rhesus macaques.

Recent work suggests that the level of virus expression and degree of immunosuppression in HIV-infected individuals may affect their ability to transmit the virus. The wives of HIV-infected hemophiliacs are at greater risk for seroconversion if the men have severe depletion of T-helper cells (19). This finding suggests that the ability of a seropositive person to transmit the virus increases with the severity of the disease, perhaps because HIV-related immunosuppression is associated with increased cell-free viremia (F. Dewolf, J. Goudsmit, D. A. Paul, and J. M. A. Lange, 3rd Int. Conf. AIDS, abstr. no. MP.53, 1987). A similar relationship between antigenemia and clinical progression of disease has also been reported with macaques infected with SIV (49). It seems likely that the level of virus present in the fluids of the reproductive tracts of humans and monkeys is related to the amount of cell-free virus in the plasma. Our inability to consistently isolate SIV from reproductive tract secretions may reflect the fact that the monkeys were usually healthy at the time the samples were collected. One positive sample from 21893 was obtained a week before the animal was euthanatized because of its deteriorating clinical condition.

The significance of sexual contact in the natural transmission of SIV is not clear, but we have shown that it is possible to transmit the virus by this route. Retroviruses other than HIV and SIV are known to be horizontally transmitted by sexual contact. Two type C retroviruses in mice (feral mouse ecotropic virus, WM-E and Friend ecotropic virus, and Friend murine leukemia virus) have been transmitted from viremic males to females by natural mating, and the viruses have been found in the semen and uterine washes from viremic animals (38).

The similarities in reproductive biology between humans and macaques and the close relationship of HIV to SIV make the model described here a valuable one for studying the sexual transmission of HIV. The model will allow careful study of mechanisms of heterosexual transmission of SIV and HIV by providing a controlled system in which a variety of factors can be manipulated. Further study will be directed toward determining the minimal infectious dose of SIV required for genital transmission and the role of cofactors in transmission.

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